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Review

Protein nanoparticle: A unique system as drug delivery vehicles

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Over the past three decades, there has been a considerable research interest in the area of developing drug delivery using nanoparticles (NPs) as carriers for small and large molecules. Targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery system. They have been used *in vivo* to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. This review presents the most outstanding contributions in the field of protein nanoparticles used as drug delivery systems. Methods of preparation of protein nanoparticles, characterization, drug loading, release and their applications in delivery of drug molecules and therapeutic genes are considered.

Key words: Nanobiotechnology, protein nanoparticles, drug delivery, release, drug loading, characterization, morphology, application.

INTRODUCTION

Controlled drug delivery technology represents one of the border areas of science, which involves multidisciplinary scientific approach, contributing to human health care. The concept of drug targeting and controlled drug delivery is used in attempts to improve the therapeutic index of drugs by increasing their localization to specific organs, tissues or cells and by decreasing their potential toxic side effects at normal sensitive sites (Dinauer et al., 2005). As in the field of cancer therapy, chemotherapeutic agents have toxic side effects for tumor cells as well as for normal cells; the controlled delivery of these agents to diseased sites would enable the use of higher doses for increasing therapeutic efficacy (Brigger et al., 2002). Controlled drug delivery involves the association of a drug with a carrier system, thereby allowing modulation of the pharmacokinetic properties and biodistribution of the drug. Different nano-sized carriers, such as nanoparticles (Leroux et al., 1995; Couvreur and Vauthier, 1991), polymeric micelles (Kataoka et al., 1993), liposomes (Bochot et al., 2002), surface-modified nanoparticles (Araujo et al., 1999) and solid lipid nanoparticles (Muller et al., 2002), have been developed and suggested for achieving these goals. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction of toxicity or side effects (Jahanshahi, 2004; Jahanshahi et al., 2005a; Jahanshahi et al., 2007a), their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, nanoparticles possess certain advantages over the liposomal delivery systems, such as greater stability during storage, stability *in vivo* after administration and ease of scale-up during manufacture (Kreuter, 1995). For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.

Therefore, the nanoparticle technology used in recent years has great promise in promoting the efficacy of drugs (Kreuter, 2001; Vijayanathan et al., 2002). Nanoparticles for the purpose of drug delivery are defined Nanoparticles were first developed around 1970. They were initially as submicron (<1 µm) colloidal particles

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(Kreuter,1991a). devised as carriers for vaccines and anticancer drugs (Couvreur et al., 1982).

They consist of macromolecular materials in which the active principle is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached (Kreuter, 1983). The body distribution of these carriers can be controlled by size and surface properties (Stayton et al., 2000). The particulate drug carrier systems are characterized by considerable load and make possible a controlled release of the drug as well as protection from degradation (Li et al., 1997).

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties (Jahanshahi et al., 2005) and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen (Soppimath et al., 2001).

The advantages of using nanoparticles as a drug delivery system include the following (Mohanraj and Chen, 2006):

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including (Kreuter, 1994): (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) Drug release profile desired; and (f) antigenicity of the final product.

Among the available potential colloidal drug carrier systems covering the size rangedescribed, protein- based nanoparticles play an important role (Kumar and Jain, 2007). Most often, serum albumin obtained from human, bovine, legumin, etc. as well as gelatin was used as the starting material for the preparations.

The present review details the latest development of protein nanoparticles drug delivery systems, its preparation methods, characterization and potential applications of nanoparticles.

PROTEIN NANOPARTICLES

The most important advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the improvement of the cellular uptake (Schafer et al., 1992) of a number of substances. As a result undesired toxic side effects of the free drug can be avoided, for example with methotrexate (Narayani and Rao, 1993).

Among of colloidal systems those based on proteins may be very capable. Proteins are a class of natural molecules that have unique functionalities and potential applications in both biological as well as material fields (Jahanshahi 2004; Jahanshahi et al., 2004). Nanomaterials derived from proteins, especially protein nanoparticles are biodegradable, non-antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. Because of the defined primary structure of proteins the protein-based nanoparticles may suggest various possibilities for surface alteration and covalent drug attachment (Weber et al., 2000).

Protein nanoparticles can be utilized for the pulmonary delivery of protein therapeutics or can be incorporated into biodegradable polymer microspheres/nanospheres for controlled release depot or oral delivery. Nowadays active research is focused on the preparation of nanoparticles using proteins like albumin, gelatin, gliadin and legumin.

Gelatin

Gelatin is one of the protein materials that can be used for the production of nanoparticles. It is obtained by controlled hydrolysis of the fibrous, insoluble protein, collagen, which is widely found as the major component of skin, bones and connective tissue (Coester et al., 2006). In terms of nanopharmaceutics, gelatin was already considered as interesting biodegradable base material in the early days of particle development (Marty et al., 1978). The interest was based on the facts that gelatin is biodegradable, non-toxic, easy to crosslink and to modify chemically and has therefore an immense potential to be used for the preparation of colloidal drug delivery systems such as microspheres and nanoparticles (Jahanshahi et al., 2008 b,c; Babaei et al., 2008). Other advantages are: it is inexpensive, can be sterilized, is not usually contaminated with pyrogens and possesses relatively low antigenicity (Schwick and Heide, 1969). Un-

fortunately, formulations containing gelatin in the outer layer (hard and soft gelatin capsules) are prone to inter or intramolecular cross-linking of gelatin with time, temperature and humidity. Because of this tendency, the very use of gelatin in pharmaceutical formulations has been put to question (Saxena et al., 2005). On the other hand, the material is used widely despite efforts to replace it with other substances (Zwiorek et al., 2004). Addition of a chemical cross-linker like glutaraldehyde, gives gelatin stability, shape and a raised circulation time *in vivo* as compared to unmodified particles (Jameela and Jayakrishnan, 1995; Jahanshahi et al., 2008b), and release is a function of cross-linking density of these nanoparticles. This structural change improves the performance, properties and characteristics of gelatin like insolubility at high temperatures, reduced swelling in water and less permeability to cell membranes (Levy et al., 1982).

Two different gelatins, A and B with different isoelectric points (IEP), are formed following either acid or base hydrolysis, respectively (Sawicka, 1990). Gelatin type A is derived from acid processed collagen, while type B is obtained by alkaline collagen treatment, resulting in a difference in isoelectric points, being 7 – 9 for gelatin type A and 4 – 5 for gelatin type B.

Characteristic features of gelatin are the high content of the amino acids glycine, proline (mainly as hydroxyproline) and alanine. Gelatin molecules contain repeating sequences of glycine, proline and alanine amino acid triplets, which are responsible for the triple helical structure of gelatin (Azarmi et al., 2006). The primary structure of gelatin offers many possibilities for chemical modification and covalent drug attachment. This can be done either within the matrix of the particles or on the particle surface only (Jahanshahi et al., 2008c). In the first case, chemical modifications have to be done to the gelatin macromolecules before nanoparticles are formed, while in the latter case the particle surface is used (Weber et al., 2005). These properties, combined with the high potential of nano-sized delivery systems make gelatin-based nanoparticles a promising carrier system for drug delivery.

Albumin

Albumin is an attractive macromolecular carrier and widely used to prepare nanospheres and nanocapsules, due to its availability in pure form and its biodegradability, nontoxicity and nonimmmunogenicity (Kratz et al., 1997). Both Bovine Serum Albumin or BSA and Human Serum Albumin or HSA have been used. As a major plasma protein, albumin has a distinct edge over other materials for nanoparticle preparation. On the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry reactive groups (thiol, amino, and carboxylic groups) on their surfaces that can be used for ligand binding and/or other surface modifications and also albumin nanoparticles offer the advantage that ligands can easily be attached by covalent linkage. Drugs entrapped in albumin nanoparticles can be digested by proteases and drug loading can be quantified. A number of studies have shown that albumin accumulates in solid tumors (Takakura et al., 1990) making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs.

Gliadin and legumin

The use of nanoparticles is of interest for bioadhesion purposes because these pharmaceutical dosage forms have a large specific surface, which is indicative of a high interactive potential with biological surfaces. For biological applications, vegetal particles have been derived from proteins (Ezpeleta et al., 1996), such as gliadin extracted from gluten of wheat and vicillin or legumin extracted from pea seeds. Their potential appears to be large, especially in the targeting of active principles.

Gliadin appears to be a suitable polymer for the preparation of mucoadhesive nanoparticles capable of adhering to the mucus layer. It has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Its hydrophobicity and solubility permit the design of nanoparticles capable of protecting the loaded drug and controlling its release (Ezpeleta et al., 1999). Gliadin nanoparticles (GNP) have shown a great tropism for the upper gastrointestinal regions, and their presence in other intestinal regions has been shown to be very low (Arangoa et al., 2001). This high capacity to interact with the mucosa may be explained by gliadin composition. In fact, this protein is rich in neutral and lipophilic residues. Neutral amino acid can promote hydrogen bonding interacttion with the mucosa whereas the lipophilic components can interact within biological tissue by hydrophilic interaction. The related protein gliadin possessing an amino and disulphide groups on the side chain has a good probability of developing bonds with mucin gel.

Legumin is also one of the main storage proteins in the pea seeds (*Pisum sativum* L.) Legumin is an albuminous substance that resembles casein and functions as the source of sulfur-containing amino acids in seed meals. The molecules of this protein have the capacity of binding together to form nanoparticles after aggregation and chemical cross-linkage with glutaraldehyde (Mirshahi et al., 2002).

PREPARATION METHODS

A lot of available macromolecules are used in preparation of nanoparticle. These macromolecules consist of proteins such as albumin, gelatin, legumin, vicillin and polysaccharides such as alginate or agarose. These substances have extensive usage in preparation of biomaterial because of their natural properties such as biodegradability and biocompatibility. Among of above

Table 1. Main methods of preparation nanoparticles from natural macromolecules (Jahanshahi, 2007).

Macromulecule	Production principle
Albumin	W/O emulsification
	Phase separation in an aqueous medium, by addition of a desolvating agent
	By modification of the pH
Gelatin	W/O emulsification
	Phase separation in an aqueous medium, by addition ofa desolvating agent
	By modification of the temperature
Vicillin, legumin	Phase separation in an aqueous medium by modification of the pH

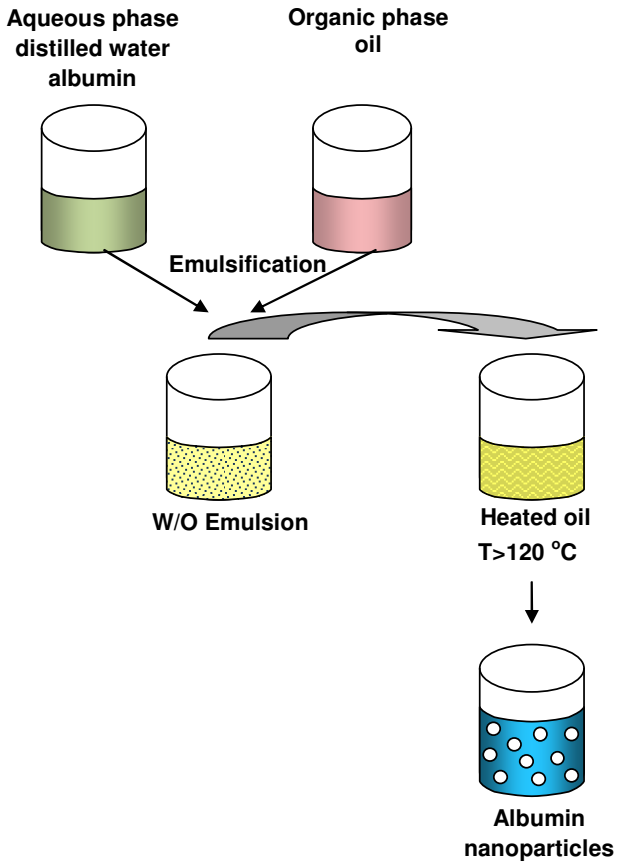

Figure 1. preparation of albumin nanoparticles with emulsification method (Jahanshahi, 2007).

mentioned macromolecules, albumin and gelatin have been used widely. Table 1 shows main methods of preparation nanoparticles from natural macromolecules. There are two basic methods for preparation of nanoparticles:

Emulsification method

Initially, its method was set forth by Scheffel and his coworkers (1972) in order to prepare albumin sphere nanoparticles and then it was optimized by Gao and his Coworkers (1995).

In this process, an aqueous solution from albumin is turned into an emulsion at room temperature and in plant oil (cotton seed oil). Then by a mechanical homogenizer with high speed, we can obtain a homogeneous emulsion. There would be a high dispersion for particles through this method. The above emulsion will be added to a high volume of pre-heated oil (over 120°C) drop by drop. This process will result a rapid evaporation of existed water and albumin irreversible destruction. This process will also cause formation of nanoparticles. The above process is shown in Figure 1. The resulted suspendsion was put into cold- ice bath.

Desolvation method

The disadvantage of the emulsion methods for particles preparation is the need for applying organic solvents, for the removal both of the oily residues of the preparation process and of surfactants required for emulsion stabilization. Therefore, as an alternative method for the preparation of protein nanoparticles a desolvation process derived from the coacervation method of microencapsulation was developed. In this method, particles in aqueous will formed by coacervation process and later on will be stabilized by cross linking agent such as glutaraldehyde.

A new method was offered by Marty and his coworkers (1978) the foundation of this method was using a desolvation factor such as natural salts or alcohol which should be added to protein solution slowly. By adding this factor, protein third structure will changed. When we have reached to a certain level of a desolvation, protein clump will be formed. In the next stage, nanoparticles will result by this polymerization clump crosslinkage with a chemical factor that is glutaraldehyde (Coester et al., 2000). In order to obtain dispersed nanoparticles not in a mass form, we must stop the system before particles start to accumulate. System turbidity will be increased owing to this desolvation factor. Particles accumulation will form alone with increasing system's turbidity. In order to stop such kind of accumulation and creating ideal nanodispersion, we must use a resolvating agent. Figure 2 shows

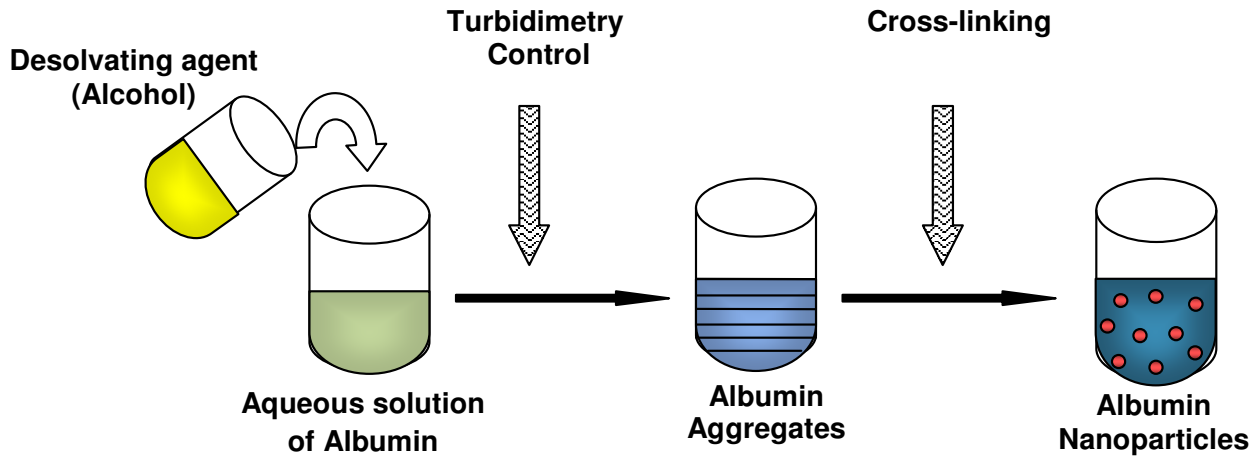

Figure 2. Preparation of albumin nanoparticles by coacervation method (Jahanshahi, 2007).

preparation of albumin nanoparticles by using desolvating agent.

CHARACTERIZATION OF PROTEIN NANOPARTICLES

Particle size

It has been shown that particle size and size distribution are the most important characteristics of nanoparticle systems (Jahanshahi et al., 2007). Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system (Panyam and Labhasetwar, 2003). Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. For example, body distribution studies have shown that nanoparticles larger than 230 nm accumulate in the spleen due to the capillary size in this organ (Kreuter, 1991b). Different *in vitro* studies indicate that the particle size also influences the cellular uptake of nanoparticles (Desai et al., 1997; Zauner et al., 2001). It was also reported that nanoparticles can cross the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may supply sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier (Kreuter et al., 2003). In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles.

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. While, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out (Redhead et al., 2001). Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability (Babaei et al., 2008). Polymer degradation can also be affected by the particle size. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size *in vitro* (Dunne et al., 2000).

Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS). PCS is industrially preferred method of sub-micron particle size analysis. The sample analyzed in the PCS device should consist of well dispersed particles in liquid medium. In such conditions the particles are in constant random motion, referred to as Brownian motion and PCS measures the speed of this motion by passing a laser. PCS determines the average particle size and Polydispersity Index (PI) which is a range of measurement of the particle sizes within measured samples. The accurate measurement of particle size must be below 0.7 (70%) (Jahanshahi et al., 2008a).

Dynamic light scattering (DLS) theory is a well established technique for measuring particle size over the size range from a few nanometers to a few microns. The concept uses the idea that small particles in a suspension move in a random pattern. Observation of larger particles compared to smaller particles will show that the larger particles move more slowly than the smaller ones if the temperature is the same.

Particle morphology

Manipulation of the physicochemical properties of materials at the nanoscale has the potential to revolutionize electronic, diagnostic, and therapeutic applications. Because of the potential large-scale use of nanomaterials, it is important to determine if there is any unique toxicity of

the nanoscale materials as compared to the bulk. It is essential for the purposes of interpreting results from cell culture and animal models that the nanomaterials are thoroughly characterized and that correlations are made between observed toxicological responses and the physicochemical characteristics of the materials. The morphology of nanoparticles was examined by two techniques. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) (Rahimnejad et al., 2006a; Rahimnejad et al., 2006). The atomic force microscope (AFM) or scanning force microscope (SFM) is a very high-resolution type of scanning probe microscope, with demonstrated resolution of fractions of a nanometer, more than 1000 times better than the optical diffraction limit.

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. SEM has the required nanometer resolution for sizing in the submicron range and is invaluable to determine the particle morphology. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

Surface charge

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation (Muller and Wallis, 1993). Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins).

Many techniques have been developed and used to study the surface modification of NPs. The efficiency of surface modification can be measured either by estimating the surface charge, density of the functional groups or an increase in surface hydrophilicity. One method used to measure the surface modification is to determine zeta potential (ξ) of the aqueous suspension containing NPs. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. The main reason to measure zeta potential is to predict colloidal stability. The interactions between particles play an important role in colloidal stability. The use of zeta potential measurements to predict stability is an attempt to quantify these interactions. The zeta potential is a measure of the repulsive forces between particles. And since most aqueous colloidal systems are stabilized by electrostatic repulsion, the larger the repulsive forces between particles, the less likely they will be to come close together and form an aggregate. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface (Mohanraj and Chen, 2006).

LOADING AND RELEASE

Drug loading

Drug may be bound to nanoparticles either (i) by polymerization in the presence of the drug- in most cases in the form of a solution(incorporation method) or (ii) by adsorbing the drug after the formation of nanoparticles by incubating them in the drug solution. Depending on the affinity of the drug to the polymer, the drug will be surface adsorbed, dispersed in the particle polymer matrixin the form of a solid solution (Harmin et al., 1986), or solid dispersion, or in some case, the drug may be covalently bound to the polymer. Therefore it is apparent that a large amount of drug can be entrapped by the incurporation method when compared to the adsorption (Breitenbach et al., 1999). The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption.

The drug loading of the nanoparticles is generally defined as the amount of drug bounded per mass of polymer (usually moles of drug per mg polymer or mg drug per mg polymer) it could also be given on a percentage basis based on the polymer.

Determination of drug entrapment

Binding of drug to the protein nanoparticles was measured by centrifuging part of the particle suspension. For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (*w*) by UV-spectrophotometry, fluorescence spectrophotometer or by a validated HPLC method. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the formulation (*W*). Effectively, (*W-w*) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given:

$$\text{Drug entrapment (\%)} = \frac{(W-w)\times 100}{W}$$

Finally, the encapsulation efficiency refer to the ratio of the amount of drug encapsulated/absorbed to the total (theoretical) amount of drug used, with regard to the final drug delivery system of the dispersion of nanoparticles.

Drug release

Release profiles of the drugs from nanoparticles depend

upon the nature of the delivery system. In the case of nanospheres, drug is uniformly distributed/dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix degradation, then the mechanism of drug release occurs mainly by diffusion, otherwise it depends upon degradation.

Many theoretically possible mechanisms may be considered for the release drug from protein nanoparticles: (a) Liberation due to polymer erosion or degradation, (b) self-diffusion through pores, (c) release from the surface of the polymer, (d) pulsed delivery initiated by the application of an oscillating magnetic or sonic field (Couvreur and Puisieux, 1993).

In many case, some of these processes may coexist, so that the distinction between the mechanisms is not always trivial. When drug release occurs by a self-diffusional process, a minimum drug loading is necessary before drug release is observed. This is easy to understand since the process involves diffusion through aqueous channels created by the phase separation and dissolution of the drug itself. This mechanism rarely occurs with drug loaded nanoparticles since, as explained before, the encapsulation efficiency of most drugs is generally too low. Infact, release from the surface and erosion or bulk polymer degradation is usually the most important processes affecting the liberation of drug from nanoparticles.

Method for quantifying drug release *in vitro* are: (i) side-by-side diffusion cells with artificial or biological membranes; (ii) equilibrium dialysis technique; (iii) reverse dialysis sac technique; (iv) ultracentrifugation; (v) ultrafiltration; or (vi) centrifugal ultrafiltration technique (Soppimath et al., 2001).

POTENTIAL APPLICATION OF PROTEIN NANOPARTICLES IN DRUG DELIVERY

The most promising areas of the application of protein nanoparticles seems to be their use as parenteral carriers for different drugs. Protein nanoparticles have been shown to enable the transport of a number of drugs across the blood-brain barrier that normally cannot cross this barrier after IV injection. The protein nanoparticle bound drugs included peptides, such as the hexapeptide endorphin dalargin and the dipeptide kyotorphin, as well as other drugs, such as loperamide, tubocurarine, doxorubicin. A number of authors have demonstrated a considerable tendency for an accumulation of protein nanoparticles in certain tumors. The binding of a variety of cytostatic drugs, 5-fluorouracil, paclitaxel (Lu et al., 2004) and doxorubicin (Morimoto et al., 1981; Leo et al., 1997) to albumin or gelatin nanoparticles significantly enhanced the efficacy against experimental tumors or human tumors transplanted to nude mice in comparison to free drug. Moreover, the toxicity of doxorubicin was substantially reduced by binding to nanoparticles.

The incorporation of magnetic particles into nanoparticles and subsequent placement of a magnetic field around the tumor is an additional way of improving the efficacy of nanoparticle–bound antitumoral drugs. Total remission of Yoshida sarcoma tumors located in the tails of rats was thus obtained with a single dose of doxorubicin bound to magnetic albumin nanoparticles targeted to the tumor by a magnetic field (Widder et al., 1983).

Antibiotics are other drugs that were shown to yield an increase in efficacy or a decrease in toxicity after binding to protein nanoparticles. Amoxicillin and gliadin nanoparticles-bearing amoxicillin (AGNP) both showed anti–*Helicobacter pylori*, but the required dose for complete eradication was less in AGNP than in amoxicillin. AGNP eradicated *H. pylori* from the gastrointestinal tract more effectively than amoxicillin because of the prolonged gastrointestinal residence time attributed to mucoadhesion. A dosage form containing mucoadhesive nanoparticles bearing a potential antibiotic should be useful for the complete eradication of *H. pylori* (Umamaheshwari et al., 2004). Another therapeutic area for protein nanoparticles is their use as carriers for ophthalmic drugs. As stated before, protein nanoparticles exhibit a considerably longer half–life in the eye than eye-drops. Pilocarpine bound to gelatin nanoparticles substantially prolonged the intraocular pressure reduction in rabbits with experimenttal glaucoma as well as the miosis time (Diepold et al., 1989) in comparison to a pilocarpine eye-drop solution. Since protein nanoparticles also adhere to inflamed ocular tissue at a level that is 4 times higher than in healthy tissue, these particles also hold promise for the targeting of anti-inflammatory drugs to inflamed sites in the eye (Das et al., 2005).

Additionally, gelatin nanoparticles is used as immunological adjuvant to enhance both humoral and cellular responses to antigen (Nakaoka et al., 1995). Many researchers have used gelatin nanoparticles as gene delivery vehicle (Truong et al., 1999). Leong and Candau (1982) used gelatin-DNA nanosphere coacervate as gene delivery vehicle to express the CFTR-gene into human tracheal epithelial cells. While Kaul and his workers (2002) used PEG-modified gelatin nanoparticles for intracellular uptake in BT/20 human breast cancer cells. Despite being used as a suitable carrier system, almost no attention has been directed towards the response of cytoskeletal organization and adhesion behaviour of cells when subjected to gelatin nanoparticles.

CONCLUSION

To the best of our knowledge, the current review is one of the first discussing potential in detail for protein nanoparticles as drug delivery system. Protein nanoparticles hold promise as drug delivery systems for parentral,

peroral and ocular administration as well as adjuvant for vaccines. Due to their greater stability and due to their easier manufacturing they offer advantages over other colloidal carriers such as liposomes and cell ghosts. The physic-chemical properties of the drug play an important role in the choice of the nanoparticle material that has to be employed. Other advances are required in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system. It can be anticipated that where large-scale fabrication of such nanoparticles is successful, the application of such delivery systems in Nanobiotechnology will contribute to de-bottlenecking of current biopharmaceutical manufacture.

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